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Review

The Fanconi anemia pathway: Repairing the link between DNA damage and squamous cell carcinoma

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ABSTRACT

Fanconi anemia (FA) is a rare inherited recessive disease caused by mutations in one of fifteen genes known to encode FA pathway components. In response to DNA damage, nuclear FA proteins associate into high molecular weight complexes through a cascade of post-translational modifications and physical interactions, followed by the repair of damaged DNA. Hematopoietic cells are particularly sensitive to the loss of these interactions, and bone marrow failure occurs almost universally in FA patients. FA as a disease is further characterized by cancer susceptibility, which highlights the importance of the FA pathway in tumor suppression, and will be the focus of this review. Acute myeloid leukemia is the most common cancer type, often subsequent to bone marrow failure. However, FA patients are also at an extreme risk of squamous cell carcinoma (SCC) of the head and neck and gynecological tract, with an even greater incidence in those individuals who have received a bone marrow transplant and recovered from hematopoietic disease. FA tumor suppression in hematopoietic *versus* epithelial compartments could be mechanistically similar or distinct. Definition of compartment specific FA activities is now critical to assess the effects of today's bone marrow failure treatments on tomorrow's solid tumor development. It is our hope that current therapies can then be optimized to decrease the risk of malignant transformation in both hematopoietic and epithelial cells. Here we review our current understanding of the mechanisms of action of the Fanconi anemia pathway as it contributes to stress responses, DNA repair and squamous cell carcinoma susceptibility.

Contents

1. Fanconi anemia pathway mutations play key role in the development of cancer

FA is a rare, autosomal recessive, and X-linked in the case of *FANCB*, syndrome characterized by congenital defects, bone marrow failure (BMF), and increased susceptibility to cancers. These are predominantly acute myeloid leukemia (AML) and head and neck squamous cell carcinoma (HNSCC) [1–4]. Disease incidence is rare, estimated at 1 in 200,000 live births, with a carrier

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frequency of 1 in 181 [5,6]. A diagnosis of FA is devastating with a median life expectancy of a little over 20 years [3,6]. Symptoms frequently occur early in life and include a constellation of birth defects, hematologic abnormalities, and cellular as well as organismal hypersensitivity to agents that cause DNA interstrand crosslinks (ICLs), such as melphalan, cisplatin, and mitomycin C (MMC). Based on the variable expressivity of recognized symptoms, it is possible that the true incidence of FA is grossly underestimated. Recommendations to screen leukemia patients who recover poorly from chemotherapy, and young squamous cell carcinoma patients, particularly those who experience serious toxicity from chemotherapy and/or radiation, have been voiced [7,8]. FA genes participate in a common pathway, which ensures genome integrity through controlling a myriad of chromatin processing and DNA damage response pathways wherein mutation of any of the FA genes individually leads to the clinical FA phenotype [9–12].

FA is described as a chromosomal instability disorder, which can affect multiple organ systems with variable severity. It is largely characterized by cellular hyper-sensitivity to DNA damaging agents that induce DNA interstrand crosslinking (ICL) [13–15], impairing DNA strand separation and unwinding, and ultimately hindering DNA replication and transcription [16,17]. Cells cultured from individuals with FA exhibit cellular accumulation in the G2 phase of the cell cycle and pronounced chromosomal breakage when exposed to crosslinkers such as mitomycin C or diepoxybutane (DEB). In fact, the formation of radial chromosomes in FA lymphocytes treated with DEB has been utilized as a diagnostic feature of FA for years [4,17–19]. Hypersensitivity to ICLs is a hallmark of FA cells, and the FA machinery was therefore initially regarded as a specialized ICL repair system reflected by the rare incidence of FA. However, the finding that the replication checkpoint kinase ataxia telengiectasia and Rad3-related (ATR) is required for triggering FA activation [20,21] and that important breast cancer susceptibility genes *BRCA2*, *PALB2* and *BRIP1* are identical to *FANCD1* [22], *FANCN* [23,24], and *FANCJ* [25–27], have re-defined the FA pathway as one of broad importance. Correspondingly, the FA pathway is activated beyond ICL by many forms of genotoxic stress including UV, ionizing radiation and oxidative stress, and FA deficient cells have been utilized as a general model system to study ATR signaling and BRCA functions in DNA repair [20,28].

FA pathway activity is tightly regulated and specifically activated during the S phase of the cell cycle [29] and in response to DNA damage [15,30]. Individuals with FA have an approximately 50-fold increased risk of developing any cancer type [2], with striking susceptibility to leukemia and squamous cell carcinomas. Carriers of *FANCD1*/*BRCA2* and *RAD51C* mutations are predisposed to breast and ovarian cancer [31–33], carriers of *FANCJ* and *FANCN* mutations are predisposed to breast cancer at lower penetrance [34–37], and carriers of *FANCD1*/*BRCA2*, *FANCC* and other FA gene mutations are predisposed to pancreatic cancer [35,38–41]. Importantly, defects in the FA/BRCA pathway exist in the general, non-FA population, and associated cancer susceptibility is thus not limited to the rare, inherited scenario. Tissue specific FA repression and crosslinker sensitivity have been reported. *FANCD2* and *FANCF* deficiencies occur in ovarian tumor cells [42–44], and *FANCF* silencing has been detected in a significant proportion of cervical, head and neck and lung cancers [45,46]. However, one report did not detect *FANCF* silencing in head and neck cancer (HNC) cells [47], and additionally, cisplatin sensitivity was not associated with the FA/BRCA pathway inactivation in HNC cell lines [48]. Similarly, Burkitt and Ljungman were unable to link cisplatin sensitivity to defective monoubiquitination of *FANCD2* in four head and neck cancer cell lines. Interestingly, however, three of four cisplatin sensitive cell lines investigated were unable to induce the formation of *FANCD2* nuclear foci, and both *FANCD2* focus formation and cisplatin resistance could be restored through the expression of exogenous *BRCA1* in these cells [49]. These findings would suggest that defects in *FANCD2* nuclear focus formation, but not ubiquitination, are indicative of cisplatin sensitivity, and that *BRCA1* deficiencies are responsible. Regardless, early identification of biomarkers for cancer cells, such as head and neck squamous cell carcinoma, which identify defects in the FA pathway may allow for alternative chemotherapeutic and/or irradiation options to be exploited. Because FA deficient cells are hypersensitive to DNA damage, low dose clastogenic treatments may be effective for sporadic SCCs with FA mutations. Systematic dose de-escalation studies in FA SCC models are needed to explore this possibility. The results may be particularly useful for FA patients where achieving tumor eradication while minimizing life threatening toxicity remains a difficult balancing act.

2. The FA pathway: multi-protein interactions coordinate DNA repair

Fifteen complementation groups and the corresponding FA genes have now been identified [14,22–24,26,27,33,50–63]. Their protein products function as either signal transducers and/or DNA processing factors within the larger FA-BRCA DNA damage response network as described below. Our published studies have demonstrated that multiple FA and associated genes are transcriptionally limiting and co-regulated through Rb/E2F pathways [64]. Upregulation of components of the DNA repair machinery in proliferating cells is likely to ensure maximal DNA repair capacity when the chance of replicative DNA damage and the need for DNA repair is greatest. The FA/BRCA pathway is activated during DNA replication and by DNA damage in the form of ICLs and other lesions. Protein components of the FA/BRCA pathway assemble into at least three complexes within the cell nucleus, these are the FA core complex (FACC), the ID complex composed of (mono)ubiquitinated FANCD2 and FANCI proteins, and a complex of the FANCN and BRCA2 proteins associated with homologous recombination that binds chromatin near DNA lesions downstream from the ID complex.

Fig. 1 depicts a working model of the FA pathway: the FA core complex is composed of eight FA proteins (FANCA, B, C, E, F, G, L, and M) along with other FA-associated proteins, such as FAAP100, FAAP20 and FAAP24 [65–69]. Multiple constituents of the core complex, as well as associated components of the pathway are phosphorylated including FANCA, G, M, D1/BRCA2, D2, and I, and these phosphorylation events are important for the repair of ICLs [70–73]. The core complex forms a nuclear, high molecular weight E3 ubiquitin ligase complex, based on the sole E3 ubiquitin ligase domain of FANCL [50]. The FANCL protein contains additional domains responsible for directing substrate binding (DRWD domain) and four E2 protein interactions (RING domain) by utilizing UBE2T [74,75]. The FANCM protein of the Fanconi anemia core complex is an important component of the pathway providing ICL resistance to cells [76]. FANCM and FAAP24, with the help of the histone-fold containing proteins MHF1 and MHF2 [77], recognize both lesions in DNA and stalled replication forks [65,78] and subsequently generate single stranded DNA [79], which is thought to activate both ATR and its downstream target Chk1 [80].

The key upstream regulators of the FA pathway, ATR and ATM kinases, are responsible for phosphorylating several components of the FA pathway and specifically direct the cellular response to DNA damage during S-phase [15,21]. The phosphorylation of FANCI has been identified as a potential molecular switch that turns on the FA pathway [81]. FANCI phosphorylation initiates the interactions between the FACC and the ID complex composed of FANCI and FANCD2 [81,82]. Following phosphorylation, both components of